Risk of Small-for-Gestational Age is Associated With Common Anti-Inflammatory Cytokine Polymorphisms

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Background: Anti-inflammatory cytokines play a key role in pregnancy maintenance. Genetic variation in anti-inflammatory cytokines could influence a woman's risk of adverse reproductive outcomes.

Methods: We investigated the relationship of polymorphisms in interleukin 4 (*IL4*), *IL5*, *IL10*, *IL13*, and transforming growth factor (*TGFβ1*) with spontaneous preterm birth and small-for-gestational age (SGA) in a nested case–control study of a prospective pregnancy cohort. Women were recruited between 24 and 29 weeks' gestation at the Wake County and University of North Carolina, Chapel Hill obstetric clinics between February 1996 and June 2000. We inferred haplotypes using the EM algorithm and the Bayesian method, PHASE. Semi-Bayesian hierarchical logistic regression was used to obtain odds ratio (OR) estimates and 95% confidence intervals (CIs) for each polymorphism.

Results: African-American mothers who carried the *IL4* GCC haplotype had greater risk of spontaneous preterm birth (OR = 2.9; 95% CI = 1.2–7.4). In white mothers, carriers of the "low-producing" *IL4* CC and *IL10* ATA haplotypes had markedly reduced risk of SGA (for the CC haplotype, 0.2 [0.0–1.2]; for the ATA haplotype, 0.5 [0.3–0.8]), whereas carriers of the "high-producing" *IL4(−589)T* variant had increased risk of SGA in both African-American and white mothers.

Conclusions: Variants related to decreased anti-inflammatory cytokine production may lower risk of SGA. Furthermore, the same mechanism that protects against SGA might increase risk of spontaneous preterm birth.

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Anti-inflammatory cytokines play a critical role in pregnancy maintenance.[1–7] From the formation of the placenta through the second trimester, the maternal–fetal interface is thought to be biased toward T-helper cell Type 2 (T$_H$2) cytokine production, which is typically anti-inflammatory. This is thought to protect the developing blastocyst by down-regulating inflammatory and cytotoxic T$_H$1-type activity until the placental barrier is established.[4,8] It has been hypothesized that preterm birth can be triggered by abnormal cytokine production favoring inflammation. Inflammation can arise in response to infection and host response, the latter being influenced by common genetic variants. Consequently, a proinflammatory environment, due either to increased production of proinflammatory cytokines or to decreased production of anti-inflammatory cytokines, could be instrumental in the etiology of preterm birth.

We investigated 12 common polymorphisms in anti-inflammatory cytokines *interleukin (IL) 4*, *IL5*, *IL10*, *IL13*, and *transforming growth factor (TGF) β1* in association with spontaneous preterm birth and small-for-gestational age (SGA) among a cohort of women enrolled in a prospective pregnancy study. The genetic variants were chosen because they are frequent (minor allele frequency greater than 5%), are known to alter the expression or function of the cytokine, or are components of known, common haplotypes. In a companion report, we describe an investigation of 10 polymorphisms in 6 proinflammatory cytokine genes on risk of spontaneous preterm birth and SGA.[9]

METHODS

A detailed description of our study design and analysis methods can be found in our companion report.[9]

Study Design

The Pregnancy, Infection, and Nutrition Cohort enrolled women between 24 and 29 weeks' gestation.[10] At the intake visit, women provided genital tract swabs, blood, and urine samples. From the larger cohort, a nested case–control sample was selected for DNA extraction, genotyping, and analysis. Of the 1202 preterm cases and randomly selected control subjects, 1138 (95%) were African-American or white, of whom 74% (n = 847) consented to genetic analyses. An additional 100 women who were neither preterm cases nor controls delivered an SGA infant. Schematics of study sample selection can be found in our companion report.[9] Characteristics of our study population can be found in Appendices 1 and 2. (All appendices are available with the electronic version of this article).

Preterm birth was dichotomized as delivery before 37 completed weeks' gestation. We defined spontaneous preterm birth as those whose clinical presentation was either premature membrane rupture or idiopathic preterm labor. Infants were classified as SGA if they fell below the 10th percentile of weight for gestational age, stratified by race, sex, and parity.

DNA Extraction and Genetic Analyses

DNA was extracted from peripheral blood samples using the ABI automated DNA extractor. Genotyping for selected polymorphisms was performed using TaqMan for allelic discrimination (Table 1).[11] A 10% random sample distributed randomly across plates was assayed in duplicate for quality control procedures.

Analytic Methods

Deviations from Hardy–Weinberg equilibrium were examined in the control series, separately by race. Linkage disequilibrium, within strata of race and case-status, was assessed for single nucleotide polymorphisms (SNPs) that occurred on the same chromosome; pairwise comparison measures were calculated in Arlequin.[12] Linkage disequilibrium was assessed for polymorphisms assuming known gametic phase using D′. D′ with an absolute value of 1 indicates loci are in complete linkage. Linkage disequilibrium also was assessed assuming unknown gametic phase using likelihood ratio tests that compared the likelihood of the sample under the hypothesis of no association between loci with the likelihood of the sample allowing the loci to be associated.[13] Haplotype frequencies were inferred for case and control groups separately as well as combined using the Excoffier and Slatkin EM algorithm.[14] Frequency distributions were then compared using an omnibus likelihood ratio test.[15] Subject-level haplotypes were reconstructed using the PHASE program.[16] We excluded subjects if 50% or more of the markers had missing genotype data, or if any of the loci-specific phase probabilities dropped below 90%. Odds ratio (OR) estimates and 95% confidence intervals (CIs) compared subjects who carried the index haplotype with all other subjects. These were estimated in 2-by-2 contingency tables stratified on maternal self-reported race.

TABLE 1. Allele Frequencies of Anti-inflammatory Cytokine Polymorphisms

SNP Identifiers			Minor Allele Frequency	
SNP Region	Alternative Identifiers	dbSNP ID	African-American	White
IL4(−1099) T>G		rs2243248	G = 0.17	G = 0.07*
IL4(−589) T>C		rs2243250	C = 0.35	T = 0.15*
IL4(−33) C>T		rs2070874	T = 0.40	T = 0.15
IL5(−746) T>C		rs2069812	C = 0.20	T = 0.30
IL10(−627) C>A	(−592)	rs1800872	A = 0.43	A = 0.25
IL10(−854) C>T	(−819)	rs1800871	T = 0.43	T = 0.24
IL10(−1117) A>G	(−1082)	rs1800896	G = 0.34	G = 0.47
IL13(IVS3–24) C>T		rs1295686	C = 0.32	T = 0.21
IL13(−1112) C>T	(−1055)	rs1800925	T = 0.40	T = 0.20
IL13(+2044) A>G	R130Q	rs20541	A = 0.19	A = 0.19
TGFβ1(+869) C>T	L10P	rs1982073	C = 0.43	C = 0.37
TGFβ1(−1347) C>T		rs1800469	T = 0.23	T = 0.29

*Borderline violation of Hardy-Weinberg Equilibrium in spontaneous preterm birth controls (*P* value 0.03–0.05).

Semi-Bayesian hierarchical logistic regression was used to obtain the OR estimate and 95% CI for the main effect of each cytokine polymorphism for SGA and spontaneous preterm birth, with separate models for African-Americans and whites.[17,18] The first-stage logistic regression model regressed spontaneous preterm birth (or SGA) on dichotomous variables for carriers of cytokine polymorphisms. Models were adjusted for smoking during pregnancy. Our second-stage model converted the β-coefficient for each polymorphism into the outcome of a linear regression, in which the regressors were the inflammatory mechanisms of the cytokine gene. A detailed description of our model construction can be found in our companion report.[9] We assumed with 95% certainty that the OR for each SNP, after adjusting for the second-stage covariates, would fall within a 10-fold range (τ^2 of 0.35). Single locus effects were also estimated in 2-by-2 contingency tables, stratified by race.

RESULTS

Analyses for spontaneous preterm birth and SGA were conducted separately because they are distinct outcomes that may be related to different environmental and genetic influences. Complete tables are available as appendices with the electronic version of this article.

Spontaneous Preterm Birth

IL4

The polymorphisms *IL4(−589)* and *IL4(−33)* are strongly linked in white individuals, with the majority of subjects carrying the CC haplotype (Appendix 3). We did not observe an elevated risk of spontaneous preterm birth associated with *IL4* variant alleles in hierarchical regression (Fig. 1 and electronic Appendix 4).

In African-American subjects, differences in haplotype frequency distributions were observed for the *IL4* haplotypes (Table 2). Subjects who carried the GCC haplotype had 2.9 times (1.2–7.4) the risk of spontaneous preterm birth compared with noncarriers. Cases and control subjects also were found to have different frequencies of the TTT haplotype (1.6; 0.8–3.3) and the TCC haplotype (0.7; 0.4–1.3). Carriers of *IL4(−33)T*, contained in the TTT haplotype, had elevated risk of spontaneous preterm birth in hierarchical regression (1.8; 0.9–3.6). Carriers of the *IL4(−1099)G* variant also had increased risk of spontaneous preterm birth (Fig. 1).

IL5 and *IL13*

Polymorphisms in the *IL5* and *IL13* genes were strongly associated with one another among both African-American and white subjects. However, African-American subjects had evidence of recombination in this region, $0.20 \leq D' \leq 0.73$. Therefore, *IL5/13* SNPs were considered as part of a common haplotype configuration in whites only. White

cases and controls had a different *IL5/13* haplotype frequency distribution. The TCC haplotype (defined by the common allele at each polymorphic loci) was more common in controls than cases. Carriers of this haplotype were at substantially lower risk for spontaneous preterm birth (0.5; 0.3–1.0; Table 3). Subjects who carried the CCT haplotype had 1.9 times (0.8–4.7) the risk of spontaneous preterm birth compared with noncarriers. However, this haplotype was relatively rare in our sample (5% overall). None of the *IL5* or *IL13* loci were important in hierarchical regression (Fig. 1 and Appendix 4).

In African-Americans, there were no important differences in haplotype frequency distributions for *IL13* (Appendix 5). However, carriers of the TCG haplotype had 2.7 times the risk of spontaneous preterm birth relative to noncarriers (1.0–7.2). Carriers of the *IL13(−1112)T* variant, contained in this haplotype, also had elevated risk in hierarchical regression (1.6; 0.8–3.1; Fig. 1).

IL10

No differences in haplotype distributions were detected between cases and controls for the *IL10* haplotype configuration in either white or African-American women (Appendices 3 and 5). Similarly, the individual *IL10* SNPs did not appear to be associated with spontaneous preterm birth (Fig. 1 and Appendices 4 and 6).

TGFβ1

No differences in *TGFβ1* haplotype distributions were detected for white or African-American subjects (Appendices 3 and 5). White women who carried the TC haplotype appeared to be at increased risk of spontaneous preterm birth (OR = 3.0; 95% CI = 0.9–9.9), which was consistent with the single locus hierarchical regression estimates (Fig. 1 and Appendix 4).

SGA

IL4

Different *IL4* haplotype frequency distributions were observed in white cases and controls (Table 4). White women who carried the CC haplotype had a substantially lower risk of SGA (0.2; 0.0–1.2). It is notable that African-American and white carriers of *IL4(−589)T* had similarly elevated hierarchical regression estimates (for African-Americans, 2.0 [0.8–4.8]; for whites, 2.0 [0.9–4.8]; Fig. 2). However, no differences in haplotype frequency distributions were detected in African-American subjects (Appendix 7).

IL5 and *IL13*

There were no differences in the haplotype frequency distributions for the *IL5/IL13* haplotype configurations in white subjects (Appendix 8), and none of the individual loci appeared to be associated with SGA (Fig. 2). In African-American subjects, the *IL13* haplotype frequency distribution

Odds Ratio
1.0

IL4(-1099)

0.8 1.6 3.0
0.6 1.3 2.8

IL4(-589)

0.5 1.1 2.9
0.4 1.0 2.5

IL4(-33)

0.9 1.8 3.6
0.4 1.0 2.5

IL5(-746)

0.4 0.9 1.7
0.4 1.0 2.7

IL10(-627)

0.4 0.9 2.2
0.4 0.9 2.3

IL10(-854)

0.4 0.9 2.2
0.4 0.9 2.3

IL10(-1117)

0.6 1.1 2.2
0.4 0.9 1.8

IL13(IVS3-24)

0.4 1.0 2.5
0.5 1.2 2.9

IL13(-1112)

0.8 1.6 3.1
0.6 1.2 2.4

IL13(+2044)

0.4 0.7 1.4
0.5 1.2 3.0

TGFβ1(+869)

0.4 0.9 1.8
0.3 0.6 1.2
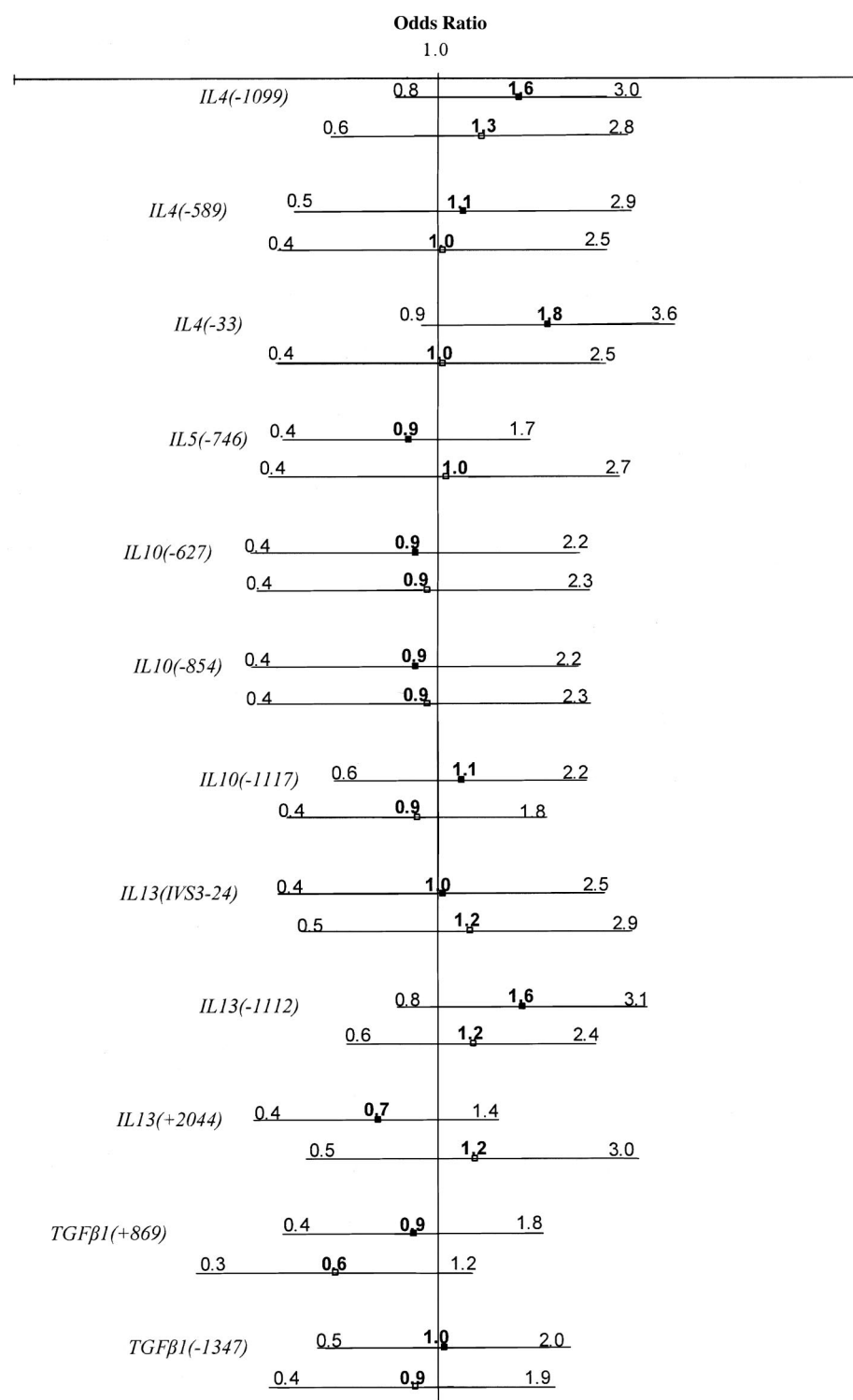
TGFβ1(-1347)

0.5 1.0 2.0
0.4 0.9 1.9

FIGURE 1. Risk of spontaneous preterm birth for anti-inflammatory cytokine polymorphisms in semi-Bayesian hierarchical logistic regression models that included both pro- and anti-inflammatory cytokine polymorphisms. The models were adjusted for smoking and the inflammatory mechanism of the cytokine and stratified by maternal self-reported race. Odds ratios and 95% confidence intervals reflect the effect of carrying a given polymorphism on risk of spontaneous preterm birth. Filled squares represent the point estimates for African-American mothers; open squares represent the point estimates for white mothers.

differed between cases and controls (Table 5). However, carriers of the haplotypes identified by permutation-based P values did not appear to be at substantially increased or decreased risk of SGA (for the CTG haplotype, 0.9 [0.4–2.0]; for the TTG haplotype, 1.3 [0.3–3.2]). Subjects who carried the *IL13(−1112)T* and *IL13(IVS3-24)T* variant alleles had increased risk of SGA in hierarchical regression (for *IL13(−1112)T*, 1.6 [0.8–3.2]; for *IL13(IVS3-24)T*, 1.6 [0.6–

TABLE 2. Haplotype Analysis for Spontaneous Preterm Birth Among African-American Mothers

IL4(−1099)	IL4(−589)	IL4(−33)	Haplotype Frequency Estimation (EM)[14]					Haplotype Reconstruction (PHASE)[16]	
			Overall*	Case	Control	χ^2	P Value†	OR‡	95% CI
G	C	C	0.09	0.15	0.07	8.44	0.01	2.9	1.2–7.4
G	T	T	0.05	0.03	0.05	11.78	0.46	0.6	0.1–2.8
T	C	C	0.26	0.18	0.29	8.43	0.01	0.7	0.4–1.3
T	T	C	0.18	0.17	0.18	6.72	0.74	0.8	0.4–1.6
T	T	T	0.38	0.46	0.36	5.43	0.04	1.6	0.8–3.3
Omnibus Likelihood Ratio test						11.80	0.05		

*Haplotypes included in table if they have a frequency of at least 5% in the African-American population. Complete tables are available from the authors by request.

†*P* values based on 10,000 permutations and represent the comparison of individual haplotype frequencies between cases and controls.

‡Odds Ratio (OR) and 95% Confidence Interval (CI) calculated using subject-level haplotype assignments from PHASE comparing carriers of a given haplotype to noncarriers of that haplotype.

4.3]; Fig. 2). Both variants travel on the identified TTG haplotype.

IL10

IL10 haplotype frequency distributions differed between white cases and controls (Table 4). Moreover, carriers of the ATA haplotype had substantially lower risk of SGA (0.5; 0.3–0.8). This haplotype contains the *IL10(−854)T* and *IL10(−627)A* alleles. These polymorphisms are tightly linked and therefore have identical hierarchical regression estimates (0.7; 0.3–1.7; Fig. 2). African-American cases and control subjects did not differ substantially with respect to haplotype frequency distributions for *IL10* (Appendix 7), though the single locus associations were consistently above 1.0 (Fig. 2).

TGFβ1

Neither white nor African-American subjects had important differences in *TGFβ*1 haplotype frequency distribu-

tions between cases and controls (Appendices 7 and 8). Carriers of individual variant alleles did not appear to be at substantially increased or decreased risk of SGA (Fig. 2).

DISCUSSION

Anti-inflammatory cytokines are postulated to contribute to the maintenance of pregnancy. Previous studies have shown that these cytokines are in high concentration, even in the first term as the placenta develops, and can play a critical role until parturition.[4,8] In contrast, an increase in the concentration of inflammatory cytokines could be an important antecedent to both term and preterm delivery (Fig. 3). This shift in the balance between pro- and anti-inflammatory cytokines may be due either to an increased production of proinflammatory cytokines or to a withdrawal of anti-inflammatory cytokine production. However, investigation of the relationship between anti-inflammatory cytokines and delivery has yielded inconsistent findings, which underscores the

TABLE 3. Haplotype Analysis for Spontaneous Preterm Birth Among White Mothers

IL5(−746)	IL13(−1112)	IL13(IVS3−24)	Haplotype Frequency Estimation (EM)[14]					Haplotype Reconstruction (PHASE)[16]	
			Overall*	Case	Control	χ^2	P Value†	OR‡	95% CI
C	C	C	0.46	0.45	0.46	2.40	0.67	0.8	0.4–1.5
C	C	T	0.05	0.09	0.04	13.92	0.07	1.9	0.8–4.7
C	T	C	0.09	0.11	0.08	9.77	0.31	1.4	0.7–2.8
C	T	T	0.11	0.16	0.10	8.02	0.08	1.6	0.9–2.9
T	C	C	0.27	0.18	0.29	6.28	0.02	0.5	0.3–1.0
Omnibus Likelihood Ratio Test:						13.19	0.03		

See Table 2 footnotes (except that first footnote refers to frequency among white women).

TABLE 4. Haplotype Analysis for SGA Among White Mothers

			Haplotype Frequency Estimation (EM)[14]					Haplotype Reconstruction (PHASE)[16]	
			Overall*	Case	Control	χ^2	*P* Value†	OR‡	95% CI
IL4(−589)	*IL4(−33)*								
C	C		0.85	0.80	0.86	1.77	0.05	0.2	0.0–1.2
T	T		0.14	0.17	0.14	5.07	0.33	1.1	0.5–2.5
Omnibus Likelihood Ratio test						9.32	0.02		
IL10(−627)	*IL10(−854)*	*IL10(−1117)*							
A	T	A	0.24	0.16	0.27	5.02	0.01	0.5	0.3–0.8
C	C	A	0.29	0.33	0.28	3.77	0.21	1.1	0.7–1.8
C	C	G	0.47	0.51	0.46	2.55	0.11	0.9	0.5–1.6
Omnibus Likelihood Ratio test						7.67	0.03		

See Table 2 footnotes (except that first footnote refers to frequency among white women).

complexity of the cytokine network. For *IL4*, one of the genes in which we observed an association with spontaneous preterm birth, the literature suggests a complex and perhaps paradoxical effect, likely reflecting both environmental and genetic factors.[19] In monocytes, IL-4 inhibits cyclooxygenase activity and decreases prostaglandin production, which is consistent with anti-inflammatory effects.[20] However, in the human amnion, chorion, and decidual cells, IL-4 enhances prostaglandin production.[21,22] This production, in combination with similar observations for IL-1 receptor antagonist (a potent anti-inflammatory cytokine) and IL-10, suggests that term labor may involve adaptive mechanisms that use circulating regulators of immune response to accelerate labor and delivery.[5,7]

IL4, *IL5*, and *IL13* are closely clustered on chromosome 5q31. IL-4 promotes T_H2 cell differentiation, and is therefore crucial to the anti-inflammatory response. The variant *IL4(−589)C* is associated with decreased transcriptional activity of *IL4* by a luciferase assay relative to *IL4(−589)T*,[23] which may ultimately result in lower levels of anti-inflammatory cytokines, shifting the balance of cytokines towards inflammation. Carriers of the GCC haplotype (which includes the low-producing variant) had almost 3-fold greater risk of spontaneous preterm birth, which is consistent with a withdrawal of IL-4 production facilitating inflammation-mediated preterm birth. Carriers of the haplotype TTT also had increased risk of spontaneous preterm birth. This haplotype has been identified as a risk factor for chronic disseminated candidiasis infection in adult leukemia patients, and for respiratory syncytial virus in children.[24–26]

The haplotype configuration that included *IL5* and *IL13* polymorphisms was associated with an increased risk for spontaneous preterm birth among whites, with those who carried the TCC haplotype being at lowest risk. Homozygotes for *IL13(−1112)T* have been found to have higher circulating

levels of IL-13.[27–30] *IL13(−1112)C* may exhibit decreased binding of nuclear proteins to the promoter region.[30] Consequently, the TCC haplotype could be associated with decreased circulating IL-13 due to the *IL13(−1112)C* variant allele; these results potentially conflict with our findings for IL-4, suggesting that decreased T_H2 cytokine production may increase the risk for spontaneous preterm birth. This highlights the complexity of variation in the T_H1/T_H2 balance.

The evidence for an association between cytokines and SGA is complex, which underscores the likelihood that multiple genes and environmental factors are involved. Both T_H2 and T_H1 favored environments have been implicated. IL-10 levels in midtrimester amniotic fluid and IL-4 levels 24-hour postpartum have been associated with SGA.[31,32] Although the IL-10 association was not confirmed in another study in humans,[33] IL-10-deficient mice produced fetal-growth-retarded offspring.[34]

The relationship between *IL4* haplotypes and SGA in whites suggests an etiology that may include an overproduction of T_H2 cytokines. In our analyses, subjects with the *IL4* CC haplotype had considerably lower risk of delivering an SGA baby; while subjects who carried the TC haplotype had considerably elevated risk. As described previously, *IL4(−589)T* is associated with increased transcriptional activity of *IL4*. Carriers of *IL4(−589)T* could have increased T_H2 activity relative to *IL4(−589)C* homozygotes given the sentinel role IL-4 plays in T_H2 cell differentiation. In fact, white carriers of the *IL4(−589)T* variant had 2-fold greater risk of SGA. While the effect of the *IL4(−589)T* allele alone in African-Americans was very similar to that in whites, the results for the same common haplotype were not similar. This difference likely reflects substantial differences in linkage disequilibrium and haplotype structure in individuals of African ancestry.[35]
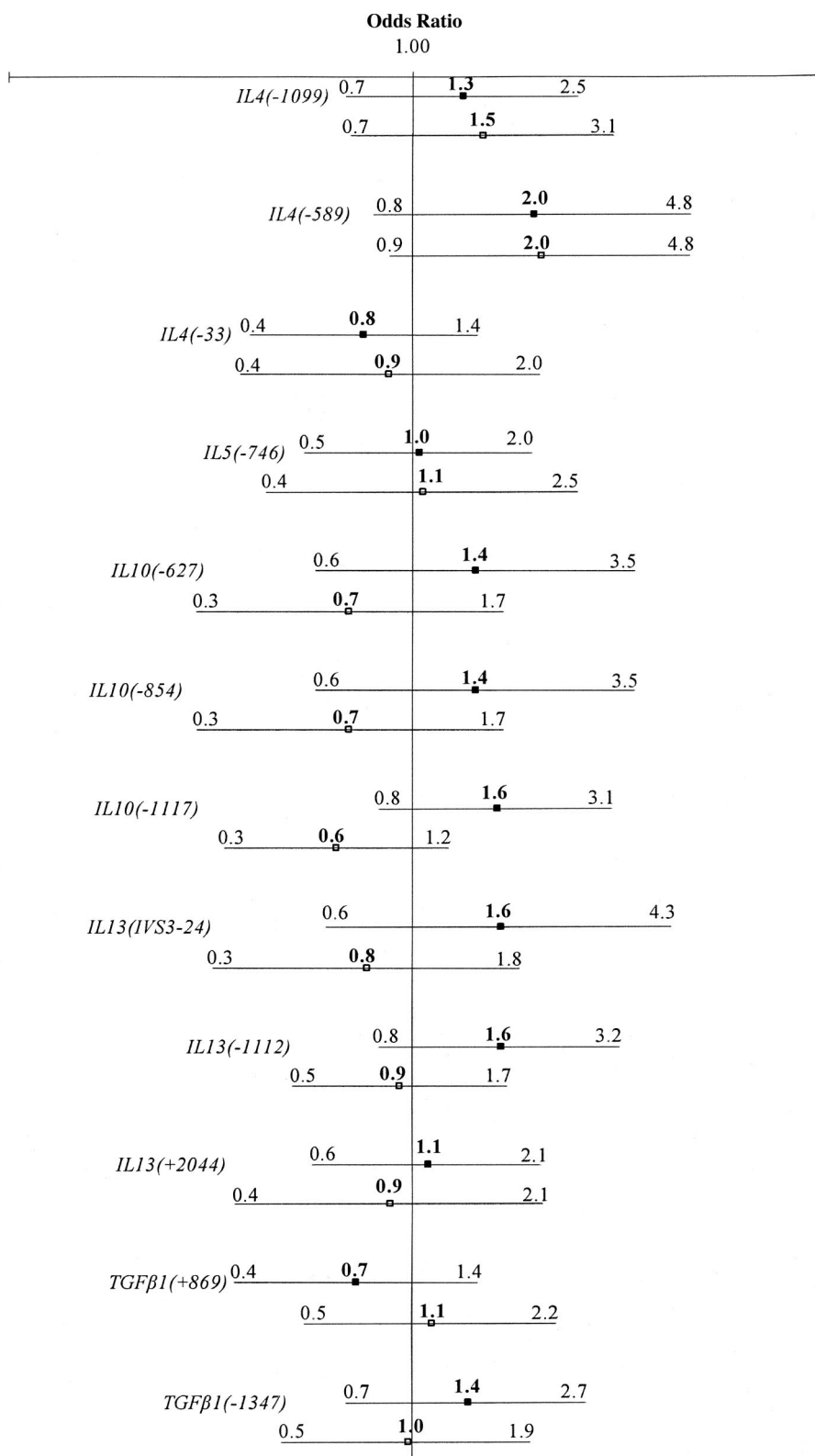
Odds Ratio
1.00

FIGURE 2. Risk of SGA for anti-inflammatory cytokine polymorphisms in semi-Bayesian hierarchical logistic regression models that included both pro- and anti-inflammatory cytokine polymorphisms. The models were adjusted for smoking and the inflammatory mechanism of the cytokine and stratified by maternal self-reported race. Odds ratios and 95% confidence intervals reflect the effect of carrying a given polymorphism on risk of spontaneous preterm birth. Filled squares represent the point estimates for African-American mothers; open squares represent the point estimates for white mothers.

TABLE 5. Haplotype Analysis for SGA Among African-American Mothers

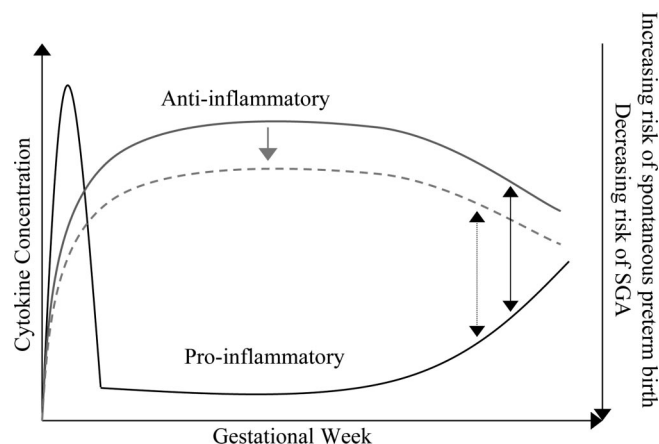| IL13(−1112)	IL13(IVS3–24)	IL13(+2044)	Haplotype Frequency Estimation (EM)[14]					Haplotype Reconstruction (PHASE)[16]	
			Overall*	Case	Control	χ^2	*P* Value†	OR‡	95% CI
C	C	G	0.27	0.25	0.29	4.95	0.36	0.6	0.3–1.6
C	T	A	0.09	0.10	0.08	7.47	0.49	1.3	0.5–3.8
C	T	G	0.25	0.18	0.27	5.14	0.01	0.9	0.4–2.0
T	T	A	0.11	0.07	0.12	6.32	0.12	1.0	0.3–3.2
T	T	G	0.25	0.37	0.21	6.66	0.00	1.3	0.3–3.2
Omnibus Likelihood Ratio test						10.86	0.04		

See Table 2 footnotes.

FIGURE 3. Conceptual model of the relationship between cytokine production and spontaneous preterm and SGA births. The relative balance between pro- and anti-inflammatory cytokines may determine the timing of delivery onset. A decrement in anti-inflammatory cytokine production may shift forward the gestational week at which that balance is achieved, possibly promoting preterm delivery. Additionally, low anti-inflammatory cytokine levels throughout gestation may reduce risk of SGA.

The risk for SGA among carriers of the *IL13(−1112)T* variant in the *IL13* haplotype configuration was different for African-American subjects. Subjects who carried the haplotype CTG had slightly lower risk and subjects who carried the haplotype TTG had slightly higher risk. *IL13(−1112)C* is associated with lower serum levels of IL-13. Therefore, subjects who carry CTG could have lower circulating levels of IL-13 relative to subjects who carry TTG, consistent with a role for increased T_H2 response in the etiology of SGA.

The *IL10* ATA haplotype has been linked to decreased transcriptional activity in the *IL10* promoter, and lower plasma levels of IL-10 in vivo, relative to GCG.[36,37] White subjects who carried the ATA haplotype were at lower risk of SGA, which

lends further support to the hypothesis that increased T_H2 activity may be crucial to the etiology of SGA. Although these findings were not confirmed in African-Americans, African-Americans have a large number of population-specific haplotypes in *IL10* and a different distribution of shared haplotypes than Hispanic-Americans or European-Americans.[35] This suggests that our marker loci were inappropriate for the African-American subgroup.

The role of cytokines in pregnancy remains uncertain. Previous studies of cytokine genes have focused on proinflammatory markers.[38–44] However, it seems clear that anti-inflammatory cytokines (which are thought to stimulate growth and development of the fetus and also limit the production of proinflammatory cytokines) could contribute to the outcome of pregnancy. Consequently, common variants in anti-inflammatory cytokine genes may be of critical importance to the outcome of pregnancy since their expression is thought to remain fairly constant throughout pregnancy.

Our data suggest that maintaining the proper balance between pro- and anti-inflammatory cytokines during pregnancy is critical to pregnancy outcome, and deviations in either direction may increase the likelihood of SGA or spontaneous preterm birth. While decreased production of specific anti-inflammatory cytokines increased risk of spontaneous preterm birth and decreased risk of SGA, increased production of anti-inflammatory cytokines increased risk of SGA. These results are consistent with a conceptual model in which the relative balance of pro- and anti-inflammatory cytokines affects pregnancy outcome (Fig. 3). The mechanism by which an overabundance of T_H2 cytokines over the course of pregnancy could lead to suboptimal fetal growth is unclear. However, our findings strongly suggest that some portion of the SGA and spontaneous preterm birth phenotypes arise from perturbations of a common cytokine network in pregnancy, with further influences from environmental challenges yet to be characterize.

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